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Concordant Clonal Delineation of Methicillin-Resistant *Staphylococcus aureus* by Macrorestriction Analysis and Polymerase Chain Reaction Genome Fingerprinting

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Pulsed-field gel electrophoresis of DNA macrorestriction fragments (macrorestriction analysis) allows epidemiologic typing and delineation of genetic relatedness of methicillin-resistant *Staphylococcus aureus* (MRSA) by indexing variations in the global chromosome architecture. Polymerase chain reaction (PCR)-mediated genome fingerprinting can also discriminate MRSA strains by detecting locally variable DNA motifs. To assess the correlation between these methods, 48 epidemic MRSA strains collected from 20 hospitals over a 10-year period were tested in a blind comparison by (i) macrorestriction analysis with *Sst*II or *Sma*I endonuclease and (ii) PCR fingerprinting with four primer sets aimed at the *mecA* gene, enterobacterial repetitive intergenic consensus sequences, and arbitrary sequences. Isolates were discriminated into 22 macrorestriction patterns and 15 PCR fingerprints. MRSA strains belonging to 12 distinct clones by macrorestriction analysis showed 11 distinct PCR genotypes distinguished by multiple band differences. In contrast, 34 of 37 MRSA strains found to be clonally related by macrorestriction analysis clustered in two highly related PCR genotypes that differed by a single DNA fragment ($P < 0.0001$). These data demonstrate concordant clonal delineation of epidemic MRSA by macrorestriction analysis and PCR fingerprinting and thereby indicate that the rapid PCR assay may be an efficient epidemiologic typing system.

Nosocomial infections with methicillin-resistant *Staphylococcus aureus* (MRSA) appear to be increasingly prevalent in many countries (5, 17). To delineate and control the transmission of epidemic MRSA strains within and among health care institutions, accurate epidemiologic typing is of primary importance. Bacteriophage typing has long been the reference method for typing *S. aureus* strains, but many MRSA strains are not typeable with the international set of phages (5, 15, 20). Therefore, molecular subtyping of MRSA has been developed. Determination of protein electropherotype (6), plasmid content and plasmid DNA restriction pattern (5, 14, 20), restriction analysis of total genomic DNA (12, 14), and restriction fragment length polymorphism generated by Southern hybridization with various DNA probes (3, 8, 10, 13, 14, 18) have successfully been used for this purpose. Recently, pulsed-field gel electrophoresis (PFGE) of macrorestriction fragments, generated by low-frequency-cleaving endonucleases that cut the chromosome at fewer than 30 sites, has provided a high level of strain discrimination among MRSA isolates of various origins (4, 8, 11, 19, 23). Furthermore, quantitative determination of macrorestriction pattern similarity allows estimation of interstrain genetic relatedness (22, 23). This can be useful for tracing the interhospital dissemination and evolution of epidemic strains over extended periods of time (22, 23).

Another newly described strategy that appears promising for intraspecies strain fingerprinting is polymerase chain reaction (PCR) amplification of hypervariable chromosomal sequences (2, 7, 9, 25–32). Random amplified polymorphic DNA analysis uses short (<10-base) oligonucleotide primers that anneal at a low stringency with multiple target sequences and that produce diverse DNA fingerprints that distinguish isolates of eucaryotic or procaryotic species (1, 21, 25, 30, 31). Another approach for PCR-mediated strain fingerprinting is based on the targeting of repeat DNA sequences with outwardly directed oligonucleotide primers (7, 25, 26, 28, 29, 32). Primers aiming at procaryotic repetitive extragenic palindromes or enterobacterial repetitive intergenic consensus (ERIC) sequences have proven to be valuable for discriminating isolates of a variety of eubacterial species, including *S. aureus* (25, 28, 29, 32). Other investigators have specifically typed MRSA strains by PCR amplification of a variable region within the staphylocoagulase gene in combination with restriction endonuclease analysis of the amplified product (9).

By resolving the bacterial chromosome into less than 30 large DNA fragments, macrorestriction analysis can detect both local sequence variation within the restriction sites and variation in global chromosome structure. PCR genome fingerprinting detects local sequence variation by probing repeat DNA motifs in the entire genome. We investigated the degree of correlation between these genotypic methods for clonal delineation of 48 well-characterized epidemic MRSA isolates collected over a 10-year period from 20 Belgian hospitals.

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MATERIALS AND METHODS

Bacterial strains. The MRSA strains (n = 48) used in the present study were of two origins. Group I included 24 isolates from patients and hospital personnel involved in a large outbreak that occurred in Erasme Hospital from 1989 to 1991. Six epidemic MRSA strains from outbreaks reported in four other Belgian hospitals between 1981 and 1991 were also part of the group I strains; these 30 strains were previously characterized by macrorestriction analysis with *Sst*II and by using other phenotypic and genotypic markers (23).

Group II was composed of a selection of 18 epidemic MRSA strains collected from 17 Belgian hospitals in 1991 and 1992 as part of a national survey conducted jointly by the Groupement pour le Dépistage, l'Etude et la Prévention des Infections Hospitalières, and the Institut d'Hygiène et d'Epidémiologie, Brussels, Belgium. These epidemic MRSA strains were selected as (i) predominant phage types associated with each hospital outbreak, as determined with the international basic set of typing bacteriophages at the routine test dilution or 100 times the routine test dilution, and (ii) representatives of all distinct clones, equated with major macrorestriction genotypes as defined previously (23), and all clonally related subtypes determined by *Sst*II and *Sma*I analyses among a collection of epidemic MRSA strains from 40 hospitals.

PFGE analysis of macrorestriction patterns. Isolation of DNA from bacterial cells lysed in agarose plugs, deproteinization, and digestion with restriction endonucleases were performed as described previously (23). Two low-frequency-cleaving enzymes were used for macrorestriction analysis, *Sst*II (5′-CCGCGG) and *Sma*I (5′-CCCGGG), both of which cleave the *S. aureus* chromosome at fewer than 20 sites. Restriction fragments were separated by PFGE at an angle of 120 degrees by using a CHEF-DR II or CHEF-Mapper System (Bio-Rad, Nazareth, Belgium) in 1% agarose gels run at 200 V in a buffer containing 44.5 mM Tris · HCl (pH 8.0), 44.5 mM boric acid, and 1 mM EDTA. Buffer was maintained at 14°C with a refrigerating unit (Mini-Chiller, model 1000; Bio-Rad). For *Sst*II digests, optimal separation was achieved by using a 2- to 8-s-pulse-time linear gradient for 12 h and then a 10- to 15-s linear gradient for 12 h; for *Sma*I digests, a 1- to 35-s-pulse-time linear gradient for 24 h was used. Molecular size markers included a bacteriophage lambda DNA concatemer (Bio-Rad) and a *Sma*I chromosomal digest of strain NCTC 8325 (4). A major macrorestriction genotype, or clone, was defined on the basis of common electrophoretic restriction patterns that differed by three or fewer fragments and that showed a similarity coefficient of greater than 85%, as described previously (23). Major genotypes were labeled by numerals, and each of their variant subtypes were indicated by a letter suffix.

PCR-mediated genome fingerprinting. Genomic DNA was isolated by the method of Jordens and Hall (12). The DNA concentration was measured by spectrophotometry at 260 nm and adjusted to 25 ng/μl in 10 mM Tris · HCl (pH 8.0)–1 mM EDTA. One microliter (25 ng) of template DNA was added to 99 μl of reaction solution consisting of 10 mM Tris · HCl (pH 9.0), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton X-100, 0.2 mM (each) deoxyribonucleotide triphosphates, and 1 U of *Taq* DNA polymerase (Sphaero-Q, Leiden, The Netherlands). The following primers were added as single species or in combination at a final total concentration of 100 pmol: (i) ERIC1R (primer 1246; 5′-GTGAATCCCCAGGAGCTTACAT-3′) (29), ERIC2 (primer 1245; 5′-AAGTAAGTGACTGGGTGAGCG-3′) (29), arbitrary primer BG2 (primer 1236; 5′-TACATTCGAGG ACCCCTAAGTG-3′) (25), and *mecA* gene 2 (primer 935; 5′-AGTTCTGCAGTACCGGATTTGC-3′) (15). These primers were selected as the most discriminatory among 13 oligonucleotide primers previously evaluated for MRSA strain fingerprinting (25). Low-stringency amplification was performed by using the previously described PCR conditions of 40 cycles of consecutive denaturation (1 min, 94°C), annealing (1 min, 25°C), and DNA chain extension (2 min, 74°C) in a Biomed model 60 thermal cycler (24). Amplimers were resolved by electrophoresis in 1 or 3% agarose gels run for 1 to 3 h at 125 V and were stained with ethidium bromide. A 75- to 12,216-bp molecular size marker (1-kb DNA ladder; GIBCO-Bethesda Research Laboratories) was used for reference purposes. Amplimer banding patterns obtained from coded DNA samples were visually interpreted by two observers who were blinded to the strain origins and PFGE patterns.

RESULTS

Macrorestriction analysis. Group I MRSA strains (n = 30) showed 12 distinct *Sst*II DNA patterns. Six of these patterns (type 1a through 1f) clustered in a clonal group of patterns, accounting for 24 isolates. Six other major types were observed among single isolates from Erasme Hospital (types 2 and 3) and from other hospital outbreaks in the period from 1981 to 1984 (types 4 through 7; Table 1).

Group II MRSA strains (n = 18) showed 13 distinct DNA types on the basis of the combined patterns obtained by *Sst*II and *Sma*I analyses. The 12 different *Sst*II patterns found among these strains are illustrated in Fig. 1A, whereas the *Sma*I patterns of the same isolates are shown in Fig. 1B. Seven *Sst*II patterns clustered at greater than 85% similarity in the same clonal group (clone 1) that was previously identified in Erasme Hospital (*Sst*II DNA types 1a, 1b, 1d, 1g, 1h, 1i, and 1j; Table 1; Fig. 1). The MRSA isolates belonging to this group also clustered into six clonally related *Sma*I patterns. One isolate showed an *Sst*II pattern with a four-fragment difference and a *Sma*I pattern with a two-fragment difference from that of type 1a, respectively, and was therefore classified in clonal group 1 (*Sst*II type 1j and *Sma*I type 1h). Five other major types were concordantly found by both *Sst*II and *Sma*I analyses in strains from other hospitals (DNA types 8 through 12; Table 1; Fig. 1).

Among all the isolates, 21 *Sst*II patterns were observed. *Sma*I analysis of group II isolates revealed a concordant classification into minor and major types, with the detection of one additional minor type related to clonal group 1, thus contributing to a total of 22 macrorestriction DNA types among the 48 isolates.

Clonal type 1 appeared to be widespread, because it included epidemic MRSA strains from three hospitals from which group I strains were obtained and from 13 hospitals from which group II strains were obtained. Table 1 summarizes the distributions of the combined groups of MRSA strains into 10 variants of epidemic clone 1 (1a through 1j) and into another 11 major types defined by *Sst*II macrorestriction analysis in comparison with the results of PCR fingerprinting.

PCR fingerprinting. PCR amplification of MRSA genomic DNAs with the four primer sets listed in Table 1 generated between 3 and 11 DNA products of 0.05 to 1.8 kb in length. The number of distinct amplimer patterns observed among the 48 MRSA isolates depended on the primer set used:

TABLE 1. Distribution of 48 MRSA strains according to *Sst*II macrorestriction genotype and PCR fingerprint by using four primers or primer combinations

Macrorestriction type (*Sst*II)[a]	PCR type[b]	Amplimer pattern with the following primer or primer combination:				No. of isolates from:	
		ERIC2	ERIC1R	ERIC2 + BG2	*mecA* + BG2	Erasme Hospital	Other hospitals
1a	I	A	A	A	A	7	2
	II	B	A	A	A	2	2
	III	A	A	A	G	1	0
1b	I	A	A	A	A	2	2
	II	B	A	A	A	1	1
1c	I	A	A	A	A	4	0
	IV	A	A	A	I	1	0
1d	I	A	A	A	A	2	2
	II	B	A	A	A	2	0
1e	I	A	A	A	A	0	1
1f	I	A	A	A	A	0	1
1g	V	A	B	A	A	0	1
1h	I	A	A	A	A	0	1
1i	I	A	A	A	A	0	1
1j[c]	I	A	A	A	A	0	1
2	VI	A	A	A	J	1	0
3	VII	A	E	A	K	1	0
4	VI	A	A	A	J	0	1
5	VIII	A	A	A	E	0	1
6	IX	H	A	A	F	0	1
7	X	G	A	A	A	0	1
8	XI	C	A	A	C	0	1
9	XII	D	A	A	H	0	1
10	XIII	E	C	B	D	0	1
11	XIV	F	A	A	B	0	1
12	XV	A	D	A	J	0	1

[a] Major macrorestriction types, or clones, are designated by numerals, and their subclonal variants (one- to three-fragment mismatch) are indicated by letter suffixes. *Sst*II analysis revealed 21 patterns; *Sma*I analysis of group II isolates provided concordant classification and revealed one additional variant pattern among clone 1 isolates.

[b] PCR fingerprinting types are designated by roman numerals, as determined by the combination of banding patterns obtained with four primers or primer combinations; each type of amplimer pattern is designated by a letter.

[c] This strain showed four and two fragment differences from type 1a by *Sst*II and *Sma*I analysis, respectively, and was therefore classified as clonally related to type 1.

ERIC2 combined with arbitrary primer BG2, 2 patterns (A and B); ERIC1R primers, 5 patterns (A to E); ERIC2 primers, 8 patterns (A to H); and *mecA2* combined with arbitrary primer BG2, 11 patterns (A to K) (Table 1).

The combination of the *mecA2* gene primer and arbitrary primer BG2 generated 8 to 10 DNA products ranging in size from <0.05 to 1.8 kb (Fig. 2). The small DNA products, which were analyzed in a separate 3% agarose gel, contributed to discrimination among patterns. Figure 3 illustrates the diversity of PCR-generated fingerprints obtained with the ERIC2 primer. Amplimer banding patterns were composed of two to three major products of 0.8 to 1.4 kb in length and up to nine minor products that produced fainter bands, which ranged in size between 0.35 and 1.6 kb. Both major and minor DNA products varied in number and size in some isolates, thus contributing to the overall discrimination among isolates. When the combination of amplimer patterns generated by all primer sets was used to define the PCR-mediated DNA type, the 48 MRSA strains were subdivided into 15 distinct types (labeled I through XV, Table 1).

Correlation between macrorestriction analysis and PCR fingerprinting. The PCR types were compared with the macrorestriction types determined by *Sst*II analysis (Table 1). General agreement was found between these two techniques, but with some discrepancies. Of 11 distinct clones defined as major macrorestriction types (clones 2 to 12) that differed by more than three restriction fragments, 10 distinct PCR types (PCR types VI to XV) were found to differ by multiple bands in the patterns produced with one to four primers or primer combinations. In contrast, of 37 MRSA isolates that were clonally related in macrorestriction type 1, 26 (70%) isolates generated an identical PCR fingerprint with all primers (PCR type I) and 8 (22%) isolates yielded a PCR fingerprint that differed from that of type I isolates by a single additional band generated with primer ERIC2 (Fig. 3, pattern B, PCR type II). The remaining three isolates of macrorestriction type 1 each showed a fingerprint distinguishable from PCR type I by a single band variation with one set of primers (Table 1, PCR types III, IV, and V). The latter variant PCR types were not correlated with minor subtypes of clone 1 defined by macrorestriction analysis with either endonuclease.

Table 2 summarizes the correlation found between classification of MRSA strains into distinct clones by macrorestriction analysis and the degree of DNA banding pattern differences produced by PCR fingerprinting. The strains classified into epidemic clone type 1 by macrorestriction analysis were more likely to exhibit a similar PCR fingerprint with one or fewer amplimer pattern differences than strains classified into distinct macrorestriction clones: 37 of 37 (100%) compared with 3 of 11 (27%) ($P < 0.0001$; Fisher exact test, two-tailed). They were also more likely to show identical PCR fingerprints: 26 of 37 (70%) compared with 0 of 11 ($P < 0.0001$; Fisher exact test, two-tailed).

DISCUSSION

Conventional *S. aureus* typing systems have major limitations, especially for MRSA strains. Phenotyping by bacteriophage susceptibility testing is hampered by the significant proportion of nontypeable MRSA strains (5, 14, 20). Antibiogram and plasmid profile determinations are limited by the instabilities of *S. aureus* plasmids, many of which frequently undergo rearrangements or are spontaneously lost or acquired (5, 14). Several genotypic methods have been applied to *S. aureus* to provide a stable means of strain identification. The restriction endonuclease patterns of total DNA or their combination with Southern hybridization with hypervariable DNA probes provides reliable strain markers for epidemiologic investigations (3, 10, 12, 14, 18, 23). However, these methods are not without pitfalls. Standard restriction of total genomic DNA produces complex electrophoretic patterns characterized by a large number of overlapping DNA bands. Therefore, only part of the restriction fragments, resolved in a narrow size window, can be used to compare fingerprints (12, 23). Southern hybridization analysis is a more cumbersome method that requires the use of multiple restriction endonucleases and the preparation of specific DNA probes for optimal discrimination (13, 14).

PFGE resolution of chromosomal macrorestriction fragments is a highly discriminatory subtyping tool that is useful for epidemiologic studies and for the delineation of genomic relatedness among MRSA strains (4, 8, 11, 19, 21, 23). This method of PFGE typing provided good results when genomic DNA was restricted with either *Sma*I, which cleaves the *S. aureus* chromosome in about 15 fragments (4, 8, 11, 21), or *Sst*II, which produces about 20 chromosomal fragments (23). PFGE was found to be more discriminating for typing MRSA strains than phage typing (23), antibiogram

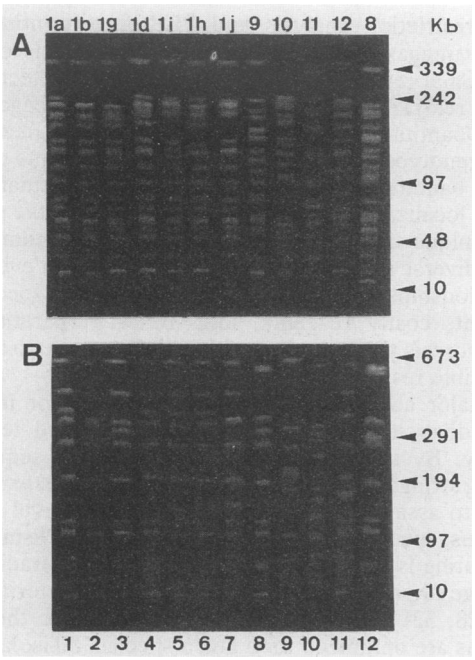

FIG. 1. PFGE patterns of DNA macrorestriction fragments of epidemic MRSA strains from diverse hospitals in 1991 and 1992. (A) *Sst*II patterns of epidemic clone 1 variant subtypes (lanes 1 to 7) and other epidemic clones (lanes 8 to 12). The *Sst*II types on the top are as described in Table 1. (B) *Sma*I patterns of the MRSA strains from panel A. Note the identical *Sma*I patterns of strains in lanes 1, 4, and 6. The molecular size scale is based on *Sma*I digestion of strain NCTC 8325 (4) and bacteriophage lambda DNA polymer.

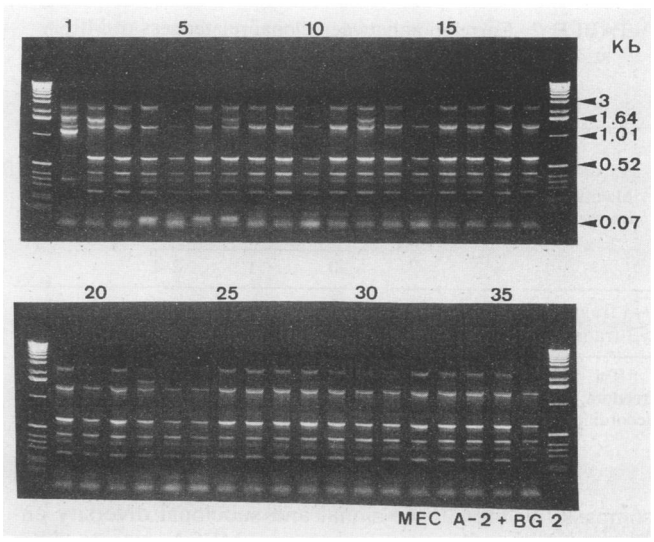

FIG. 2. Representative MRSA genomic DNA amplimer patterns produced by PCR with the combined *mecA* and BG2 primers as resolved in a 1% agarose gel. Lanes 1 to 8, single isolates with patterns D, E, F, B, G, C, H, and I, respectively (some of which were distinguished by lower-molecular-mass band differences that were more clearly visible on a 3% agarose gel [data not shown]); lanes 12 and 22, isolates with pattern J; other lanes, isolates with pattern A.

determination (11, 23), conventional restriction endonuclease analysis (23), and ribotyping (19). PFGE was also more discriminating than plasmid profile analysis in some (8, 11), but not all (4), studies.

Detection of genomic polymorphism can also be achieved by a variety of PCR methodologies (2, 7, 9, 21, 22, 25–32). Low-stringency PCR with random oligonucleotide primers generates polymorphic DNA products from diverse species of eucaryotic and procaryotic organisms (2, 21, 25, 30, 31). Other primers designed to amplify DNA regions between moderately repetitive elements such as repetitive extragenic palindromes or ERIC sequences are also capable of identifying interstrain genotypic diversity and therefore appear to be applicable to epidemiologic typing (22, 25, 26, 28, 29, 32).

One approach to PCR typing of MRSA has been developed by Goh and colleagues (9), who amplified a variable sequence of the staphylocoagulase gene by using the nested primer technique and determined its restriction fragment length polymorphism by *Alu*I digestion. The fingerprints thus obtained delineated *S. aureus* strains in a manner that correlated well with multilocus enzyme electropherotype and epidemiologic data (9). Very recently, Saulnier and coworkers (21) compared random amplified polymorphic DNA typing by using three short arbitrary primers with PFGE typing by using *Sma*I for the discrimination of MRSA strains. They noted that macrorestriction with this enzyme was more discriminating than the combined PCR fingerprints obtained with the three primers (21). Using a simple PCR assay, van Belkum and coworkers (25) screened 13 primers and additional primer combinations for optimal MRSA strain discrimination. These primers are based on *mecA* sequences, DNA repeat sequences, and arbitrary sequences of 10 to 22 nucleotides in length. Four primers or combinations of primers that achieved the highest resolution yielded 23 different genotypes among 48 sporadic MRSA isolates collected from patients in two health care institutions. These PCR types correlated well with the phage typing results obtained by using international and supplementary bacteriophage sets (25).

In the present study, the same four primers were used under identical assay conditions to produce PCR fingerprints among 48 epidemic MRSA strains from 20 hospitals. These strains were selected on the basis of their genotypes determined by *Sst*II and *Sma*I macrorestriction analysis to en-

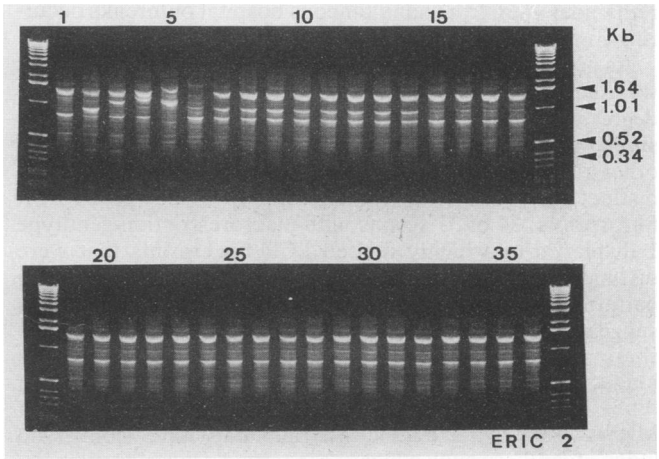

FIG. 3. Representative MRSA isolate amplimer patterns produced by PCR with the ERIC2 primer and separated in a 1% agarose gel. Lanes 1 to 6, single isolates with patterns F, C, D, G, E, and H, respectively; lanes 7 to 14, isolates with pattern B; lanes 15 to 36, isolates with pattern A.

TABLE 2. Correlation between clonal relatedness of MRSA strains determined by macrorestriction analysis and PCR fingerprinting on the basis of four primer-specific amplimer patterns[a]

Macrorestriction pattern divergence (classification)	No. of MRSA strains with a PCR fingerprint different by the following no. of amplimer patterns:			Total no. of strains
	0	1	2–4	
0–3 fragments (subclones)	26	11	0	37
>3 fragments (clones)	0	3	8	11

[a] The extended Mantel chi-square for linear trend was 29.9 (1 degree of freedom; $P < 0.0001$) for the distribution into distinct macrorestriction clones according to the degree of PCR fingerprint divergence.

compass the breadth of clonal and subclonal diversity encountered in recent years among MRSA strains from Belgium and to include a set of homogeneous MRSA isolates with identical PFGE patterns collected from one hospital. The concordance in delineating closely related from less related strains by these two methods further validates the reliability of this PCR fingerprinting method. Overall, 15 PCR genotypes were recognized on the basis of the combined amplimer patterns, whereas 22 macrorestriction patterns were recognized. Specific primer combinations produced a diversity of amplimer patterns that was similar to that observed in other MRSA isolates (25), apart from the ERIC2-BG2 primer combination, which, for reasons that are unclear, produced less diverse fingerprints in the present study. Fewer PCR types were found in the present investigation than were observed in the previous study (25) among a similar number of isolates by using the same primers and PCR assay conditions. Likely explanations for this difference include the different criteria used for selecting isolates in each study and the different epidemiologic backgrounds of MRSA occurrence in The Netherlands and Belgium. In the previous study (25), randomly selected clinical MRSA isolates were used to assess the discrimination of PCR types, and these isolates were presumably derived from sporadic cases of infection. In contrast, most isolates analyzed in the present study were from one hospital epidemic and the remainder were from simultaneous hospital outbreaks occurring in neighboring regions.

As noted before (21, 25), a core of comigrating DNA bands was shared by a majority of PCR genotypes, indicating some degree of overall relatedness among these MRSA strains. A similar observation was made in studies of MRSA by using other genotypic methods (4, 14, 23). Whereas strains with 11 distinct major macrorestriction genotypes showed 10 PCR fingerprints, 34 of 37 strains with macrorestriction genotype 1 displayed two highly related PCR fingerprints that were distinguished by a single-fragment variation in the banding pattern generated with one of the four primers. These data suggest that many epidemic MRSA strains from Belgium share a conserved genome structure, on the basis of both long-range macrorestriction mapping and PCR probing of repeat DNA motifs. Because it appears highly unlikely that MRSA isolates of distinct origins may have converged independently to such a highly conserved genomic structure, it can be inferred that these strains are the progeny of a recent common ancestor strain. Thus, these combined genotypic data provide strong evidence of clonal dissemination of MRSA to several Belgian hospitals.

Macrorestriction analysis and PCR fingerprinting each have advantages and limitations. PFGE analysis of large chromosomal restriction fragments accurately detects chromosomal rearrangements, even within related lineages (1, 4, 22–24). Quantitative estimates of the genomic relatedness of distinct genotypes can be derived from coefficients of similarity of banding patterns (23). Coupled with analysis of fragment localization of mapped genes with suitable probes, this technique can be used to compare the chromosomal maps of diverse strains and examine directly their evolutionary relationships (4). However, PFGE requires specialized equipment, costly reagents, long DNA preparation and electrophoresis times, and considerable expertise to achieve reproducible results.

The major advantages of PCR technology for indexing genome diversity are its utmost versatility and technical simplicity. By selecting the appropriate target sequences, annealing stringency, and DNA products to be analyzed, it is possible to assay conserved sequences at the level of phylum, genus, species, strain, or gene allele (7). PCR-mediated fingerprinting is being applied to epidemiologic tracing of a wide range of pathogens from viruses (16) to bacteria (9, 21, 22, 25, 28, 32) to opportunistic fungi (2). Once the assay conditions are optimized for a given species, 50 isolates can be analyzed in 48 h. Given the increasing use of PCR technology for diagnostic applications, the necessary equipment should become widely available in microbiology laboratories. Amplified polymorphic DNA patterns detect locally variable DNA motifs and can be analyzed quantitatively to study strain relationships (21, 32).

The current limitations of PCR genome fingerprinting include its limited discriminatory power compared with that of PFGE typing (21, 22). We believe, however, that further optimization of discrimination may be achieved by using additional primers and/or modified PCR assay conditions (25). PCR fingerprints were somewhat difficult to compare visually because discriminating DNA bands were often weakly stained, minor PCR products, as noted from the results of the random amplified polymorphic DNA assay (21). Finally, the reproducibility of PCR-mediated patterns is critically dependent on assay conditions and reagents. Using previously optimized PCR conditions, reproducibility was good (25). However, a strict adherence to the DNA isolation protocol and a standardized target-primer DNA ratio was needed to reproduce the DNA banding patterns observed for a given isolate on repeat testing (25). Furthermore, we noticed that batch-to-batch variations in the primers used for PCR may lead to alterations in amplimer banding patterns (27). These artifacts do not, however, influence the outcomes of interstrain comparisons, since concomitant changes occur for all isolates grouped in the same PCR type on the basis of prior experiments. In the present study, all isolates were typed in one run by using a single batch of reagents.

In conclusion, the results of the present study indicate a strong correlation between clonal delineation of MRSA isolates by macrorestriction analysis and PCR fingerprinting, as we observed in similar comparisons applied to other bacterial species (22, 28). These findings further validate the epidemiologic applicability of a simple and rapid PCR procedure with four primer combinations and gel electrophoresis analysis of amplimer patterns. Macrorestriction analysis and PCR-mediated fingerprinting are independent approaches that can be used to explore complementary levels of bacterial genome diversity. Although more difficult to perform, PFGE of macrorestriction fragments appears to be

the most discriminating method for typing MRSA strains which are of a clonal nature (11, 19, 21, 23). Clonal dissemination of MRSA to several Belgian hospitals was documented by both genotypic methods. The origin, extent, and mode of dissemination of this epidemic clone are being investigated in a national study aimed at providing clues for effective control. It is expected that the application of the molecular genotyping procedures reported here will provide further insight into these questions.

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